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Buprenorphine (Bup) is the most commonly used analgesic in mice, yet few objective assessments address its superiority for postsurgical recovery. In mice, IP implantation of a radiotelemetry device induces decreases in body weight (BW), food and water intake (FI, WI), core temperature (Tc), and activity levels that persist approximately 14 d in the absence of analgesia. To compare the efficacy of Bup with that of the nonsteroidal antiinflammatory drug indomethacin (Indo) for postsurgical recovery, male C57BL/6J mice were treated on the day of radiotelemetry implantation with Bup (0.3 mg/kg SC) or Indo (1 mg/kg SC) followed by treatment with Indo (1 mg/kg PO) on the next day (Bup-Indo versus Indo-Indo). Responses were compared between treatments in mice implanted with a radiotelemetry device and those that did not undergo surgery. Changes in BW, FI, WI, Tc, and activity were examined throughout 14 d of recovery. Indo-Indo was more efficacious in inhibiting postsurgical BW, FI, and WI reductions, compared with Bup-Indo. Bup also reduced BW and FI in the absence of surgery, indicating a nonspecific effect of this drug on these variables. Indo-Indo treatment was associated with higher activity levels during lights-on-to-lights-off transition periods compared with that observed with Bup-Indo. According to 5 objective measures of surgical recovery, our data suggest that Indo-Indo treatment is more efficacious than is Bup-Indo for postsurgical recovery of radiotelemetry-implanted mice.

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Effects of Indomethacin and Buprenorphine Analgesia on the Postoperative Recovery of Mice

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Buprenorphine (Bup) is the most commonly used analgesic in mice, yet few objective assessments address its superiority for postsurgical recovery. In mice, IP implantation of a radiotelemetry device induces decreases in body weight (BW), food and water intake (FI, WI), core temperature (T_c), and activity levels that persist approximately 14 d in the absence of analgesia. To compare the efficacy of Bup with that of the nonsteroidal antiinflammatory drug indomethacin (Indo) for postsurgical recovery, male C57BL/6J mice were treated on the day of radiotelemetry implantation with Bup (0.3 mg/kg SC) or Indo (1 mg/kg SC) followed by treatment with Indo (1 mg/kg PO) on the next day (Bup–Indo versus Indo–Indo). Responses were compared between treatments in mice implanted with a radiotelemetry device and those that did not undergo surgery. Changes in BW, FI, WI, T_c, and activity were examined throughout 14 d of recovery. Indo–Indo was more efficacious in inhibiting postsurgical BW, FI, and WI reductions, compared with Bup–Indo. Bup also reduced BW and FI in the absence of surgery, indicating a nonspecific effect of this drug on these variables. Indo–Indo treatment was associated with higher activity levels during lights-on–to–lights-off transition periods compared with that observed with Bup–Indo. According to 5 objective measures of surgical recovery, our data suggest that Indo–Indo treatment is more efficacious than is Bup–Indo for postsurgical recovery of radiotelemetry-implanted mice.

Abbreviations: Bup, buprenorphine; BW, body weight; Dex, dextrose; FI, food intake; Indo, indomethacin; NSAID, nonsteroidal antiinflammatory drug; WI, water intake; SCarb, sodium carbonate; T_c, core temperature

The *Guide for the Care and Use of Laboratory Animals* (The *Guide*) states that the alleviation of pain is a humane and ethical obligation that is an important aspect of the care and welfare of laboratory animals.[26] As such, an integral part of experimental animal welfare should be the alleviation of postsurgical pain and distress through the administration of analgesics. A survey of UK veterinarians showed that prior to 1999, only 25% of rodents received postoperative analgesia.[6] This statistic is a serious concern, because several studies have shown the negative effect of surgical procedures on behavioral and physiologic homeostasis in small rodents.[14,19,21] Surgery also may compromise experimental results if sufficient recovery time is not factored into the protocol design. Therefore, animal investigators have a legal and ethical responsibility to provide and assess pain relief in animal subjects.[20]

One of the caveats of rodent pain research is the difficulty in adequately assessing pain and distress in prey species that effectively conceal outward signs of discomfort; visual observation does not provide an accurate assessment of pain and discomfort in these species. The International Association for the Study of Pain (IASP) has published guidelines for effective detection of pain.[10] This association recommends a 2-step process in which physiologic (stress-related protein levels) and behavioral (sleep patterns) responses initially are compared between experimental and control animals in the untreated condition and then are reassessed after analgesic treatment. A comparison of results between these 2 conditions permits assessment of the degree of pain and distress in the animal.[10] Although potentially useful for the study of pain, this procedure is not easily implemented in a laboratory setting, because it may interfere with experimental protocols that are not specifically designed to study pain.

Compliance with the ethical obligations of rodent pain relief likely would be improved if objective measures were identified that could be readily implemented in a laboratory setting with little or no stress to research animals. Traditionally, measurements of pain relief have involved procedures such as the tail-flick test, in which the time to latency of withdrawal of the tail after exposure to a noxious heat stimulus is compared between analgesic-treated and untreated animals; prolongation to withdrawal from the stimulus after analgesia treatment is provided as an objective measure of pain relief.[3,24,34] However, these experimental procedures typically are confounded by handling or restraint requirements that may affect the animal and complicate data interpretation. For example, handling and restraint are well known to induce hyper- or hypothermia (that is, unregulated changes in core temperature) that stimulate compensatory changes in blood flow to the tail (a key thermoregulatory organ of rodents) that can alter the tail-flick response.[9,11] In addition, with respect to surgical recovery, pain is only 1 component of a complex set of physiologic variables that are altered after surgery; therefore, apparent recovery improvement should not be attributed solely to a decrease in pain especially since pain is not always directly measurable.

In recent years, the number of surgical procedures in small rodents has surged, with few advancements in our understanding of postoperative pain and analgesic requirements in these species. Radiotelemetry is a recent technologic advancement that is commonly used for the study of thermoregulatory and behavioral responses in mice. Radiotelemetry is a powerful technique as it permits the remote sensing of a variety of physiologic (for example, core temperature, cardiac electrical activity) and behavioral (for example, locomotor activity) measures in

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conscious, freely moving animals. The main advantage of this technology is the elimination of experimental stressors, such as restraint and handling that typically are associated with the use of rectal probes for core temperature (T_c) measurements. The main disadvantage of this technology is the requirement for an invasive surgical procedure (that is, laparotomy) and the discomfort of the device, as the transmitter typically is implanted in the peritoneal cavity and may represent as much as 15% of the body mass of a mouse.[19] Laparotomy is an invasive surgical procedure that can induce profound decreases in body weight (BW), food intake (FI), and water intake (WI) in small rodents; these effects are thought to be due in large part to the pain and distress induced by the incisional wound. Intraperitoneal implantation of a radiotelemetry device introduces an additional stress, proportional to the size of the implant, because it may compress the internal organs of small rodent species, such as the mouse. In the absence of analgesia, mice may require as long as 14 d for the reestablishment of presurgical BW after radiotelemetry transmitter surgery.[19] Guidelines are not available for efficacious analgesia for the alleviation of postsurgical pain and distress in radiotelemetry-equipped mice.

The analgesic most commonly used in rodent research is buprenorphine (Bup), a partial μ-receptor opioid agonist that is related to, yet more potent than, morphine. Buprenorphine has shown analgesic efficacy in rat and mouse models of acute and chronic pain,[3] but despite its common use as an analgesic for rodent surgeries, current data validating the physiologic actions of this drug with respect to objective measures of pain or surgical recovery are insufficient. When a behavior-based scoring system was used to assess both the duration of pain in rats that had undergone laparotomy and the efficacy of several analgesics (buprenorphine, ketoprofen, and carprofen),[27,29] surgical recovery was dependent not only on the nature of the surgical procedure but on the type of analgesic, route of administration, and dose. In addition, nonspecific effects, such as reduced activity and exploratory behaviors, were so pronounced with buprenorphine as to make the behavior-based assessment of its analgesic effects impossible. In several other studies, multiple doses of buprenorphine injected SC reduced food consumption and BW of mice and rats compared with untreated animals or those given a single buprenorphine dose.[7,13,31] Another study that showed nonspecific buprenorphine effects[12] examined analgesic efficacy after abdominal surgery in mice treated with several analgesics. Mice received acetaminophen (320 mg/kg), ibuprofen (40 mg/kg), or an acetaminophen–buprenorphine combination (640 and 10 mg/kg, respectively) in the drinking water, with a fourth group treated with IP injection of buprenorphine (2.4 mg/kg), which was delivered after surgery and again 16 h later. Ibuprofen-treated mice recovered WI and activity levels more rapidly than did untreated mice, whereas the buprenorphine injections induced several undesirable side effects including hyperactivity, hyperthermia, and reduced FI and WI compared with those in saline-treated mice. Despite these confounding influences, buprenorphine continues to be widely used subcutaneously and orally by laboratories performing invasive surgical procedures in rodents.

Another general class of analgesics is nonsteroidal antiinflammatory drugs (NSAIDs; for example, ibuprofen, indomethacin). NSAIDs represent an attractive class of drugs because of their relatively low toxicity and ready availability as over-the-counter treatments.[1] Although these compounds have previously been thought to be weak analgesics, new types of NSAIDs have been developed with increased inflammatory and analgesic potency. Although this class of drugs has been associated with various side-effects such as gastrointestinal disturbances (ulceration, hemorrhage), these are rarely a problem with short-term use (for example, 2 to 3 d).[5] An additional advantage of NSAIDs is the ability for oral administration in the drinking water or food, which permits voluntary consumption (opiates typically require injection) and eliminates the need for animal handling during the postsurgical period of wound healing.[25]

Currently, clear guidelines are not available with respect to the most appropriate postsurgical pharmaceutical regimen and objective measures of postsurgical recovery in mice that have undergone transmitter implantation. With the increasing use of implantable instrumentation in mice comes a humane and ethical obligation to develop analgesic guidelines that provide for the relief of pain while supporting surgical recovery. Therefore, the objective of this study was to compare the effectiveness of Bup (opiate; day 0)–Indo (NSAID; day 1) versus Indo (NSAID; day 0)–Indo (NSAID; day 1) on surgical recovery of mice after IP implantation of a radiotelemetry device. We hypothesized that the Indo–Indo treatment regimen would be more efficacious than Bup–Indo for postsurgical recovery of mice due to the enhanced antiinflammatory properties of the Indo–Indo protocol. Our choice of the NSAID indomethacin for this study was based on the following: (1) previous studies showed a lack of an effect of indomethacin (and its vehicle control) on circadian T_c and activity levels in mice, suggesting the absence of nonspecific effects in this species;[15,16] (2) indomethacin can be administered orally, eliminating the need for an injection procedure (decreased handling and stress of a surgically implanted animal); and (3) indomethacin is an NSAID that does not profoundly inhibit platelet aggregation, thus minimizing postsurgical bleeding complications (for example, aspirin is a strong antiplatelet-aggregating agent).

To determine the efficacy of Bup–Indo versus Indo–Indo with respect to surgical recovery, we assessed the time required for C57BL/6J male mice to reestablish presurgical levels of BW, FI, and WI in conjunction with the display of a robust circadian T_c and motor activity profile after IP implantation of a radiotelemetry device that represented approximately 14% BW.

Materials and Methods

Animals. Fifty-nine specific pathogen-free male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were used. All mice were verified free of contagious ectoparasites, helminth endoparasites, and antibodies to 17 murine viruses prior to shipping. After arrival at our facility, we did not perform routine serologic monitoring, nor did we introduce animals from other sources into this colony. Mice were housed individually in polycarbonate cages (11.5 × 7.5 × 5 in.) fitted with HEPA-filter cage tops and woodchip bedding (Pro-Chip, PWI, Canada). Rodent laboratory chow (LM-485, Harlan Teklad, Madison, WI) and water were provided ad libitum under standard laboratory conditions (25 ± 2 °C; 12:12-h light:dark cycle; lights on at 0700). Fresh cages, food, and water were provided on a weekly schedule. In conducting research using animals, we adhered to the *Guide for the Care and Use of Laboratory Animals* in an AAALAC-accredited facility. All procedures received institutional animal care and use committee approval before experiment initiation.

Environmental enrichment. The housing of laboratory mice in barren environments, such as standard plastic laboratory cages, is well known to induce the expression of abnormal behaviors, known as stereotypies (that is, invariant, rhythmic, and repetitive behaviors with no apparent function).[37] To minimize the expression of stereotypies in this study, environmental enrich-

ment was provided through the insertion of a Mouse House (Nalgene Nunc, Rochester, NY) in each home cage; this product is a red plastic triangular insert that provides a dark enclosure and climbing apparatus for small rodents. A maplewood enrichment product (W0002, Bio-Serv, Frenchtown, NJ) also was provided in each cage and impregnated with a food treat on animal arrival to encourage foraging behavior.

Treatment groups. On arrival, mice were matched for BW and then randomly assigned to treatment groups (Table 1). The surgical and nonsurgical groups represent those mice treated with vehicle or analgesic after IP implantation of a radiotelemetry device or in the absence of surgery, respectively. The nonsurgical groups were included to determine the effect of the vehicle or analgesic solutions on BW, FI, and WI in the absence of an invasive surgical procedure (that is, nonspecific effects unrelated to pain). In surgical groups, the vehicle or analgesic solution was injected SC (26-gauge needle, less than or equal to 0.30 ml/mouse) into each anesthetized mouse immediately after transmitter surgery, followed by oral dosing of conscious animals at 0900 the next day. In nonsurgical treatment groups, mice were anesthetized for 15 min (the average time of anesthesia during transmitter surgery) and then injected SC with the vehicle or analgesic and dosed orally the following day at 0900. To minimize the number of mice required in the control experiment, each animal in the nonsurgical groups served as its own control, receiving the analgesic treatments during week 1 and vehicle treatments during week 2.

Oral dosing was achieved by pipetting an equivalent drug dose (approximately 70 µl) of the vehicle or analgesic solution onto 1/2 (approximately 0.5 g) of a pina colada-flavored treat (F05475-1, Supreme Mini Treat, Bio-Serv, Frenchtown, NJ; Table 1 and Figure 1). Each treat was prepared with the vehicle or analgesic solution approximately 18 h prior to oral dosing; on the day after surgery the treat was placed on the bottom of the cage for voluntary consumption, which was visually verified to occur within 1 h of placement in the cage for each animal. Therefore, this method of analgesic administration served as an effective oral delivery system and eliminated the need for handling and injection procedures the day after surgery.

Surgical procedures. All surgical procedures were performed on day 0 (approximately 0830 to 1400), with groups alternated sequentially throughout the day to minimize potential circadian effects on anesthesia, analgesia, and recovery variables. The fact that all the surgical procedures cannot be performed at the same time is inescapable; therefore, we used a sequential design in which an animal from each treatment group was implanted sequentially, and this pattern was repeated throughout the day. This experimental format was designed to 'wash out' the time of surgery as a potential factor affecting recovery, particularly with respect to circadian T_c and activity rhythms. By using this design, 1 animal from each of the 4 treatment groups (described in detail later) was implanted each hour (that is, approximately 15 min per surgery), with surgery conducted on 2 consecutive days to achieve a sufficiently large sample for statistical comparisons among groups.

Each mouse was implanted IP with a battery-operated, free-floating transmitter (model TA10TA-F20, Data Sciences International, St Paul, MN) with a weight of 3.5 g and volume of 1.75 ml. BW was approximately 25.5 g for all mouse groups on the day of surgery (approximately 3 1/2 wk after arrival); this weight was greater than the manufacturer's recommended nominal BW of 20 g for implantation of this transmitter model. The transmitter represented approximately 14% of BW; due its large size, the transmitter could not move throughout the peritoneal cavity and, at necropsy, typically rested among the folds of the small intestine. The details of the effect of this transmitter size on mice have been previously described.[19]

Mice were anesthetized with isoflurane (induction, 2.5%; maintenance, 1%; 100% O_2; flow rate, 0.5 l/min) for surgical implantation of the radiotelemetry device. Surgical preparation consisted of shaving the abdominal fur and scrubbing the shaved area with a 10% povidone–iodine solution (Betadine Solution, Purdue Frederick, Stamford, CT) followed by 70% isopropyl alcohol. An incision (approximate length, 1 cm) was made through the skin and abdominal muscle layer by using aseptic technique. Each transmitter was disinfected by presoaking for at least 1 h in chlorhexidine diacetate (Novalsan, Fort Dodge Animal Health, Fort Dodge, IA) followed by a rinse in 0.9% sterile saline solution prior to placement in the peritoneal cavity. The peritoneal muscle layer was closed with interrupted sutures, and the skin layers were closed with continuous subcuticular sutures (4-0 silk, Ethicon, Somerville, NJ). Immediately after surgery (or 15 min of anesthesia in nonsurgical groups), each mouse was injected SC with the appropriate vehicle or analgesic solution and then returned to its homecage with ad libitum food and water. The vehicle and analgesia dosing regimens are described in Table 1.

Drugs. Indomethacin (I8280, Sigma, St Louis, MO) was prepared fresh in 0.01 M sodium carbonate (SCarb, pH 7.2). The stock solution of indomethacin was 0.001 M (0.3578 mg/ml), which was diluted 4-fold (0.00025 M) for injection (1.0 mg/kg, less than 0.3 ml/mouse) or used undiluted for oral dosing. Buprenorphine (NDC 12496-0757-1, Buprenex, Reckitt and Colman Pharmaceuticals, Richmond, VA) was stored in ampules as a stock solution (0.3 mg/ml) that was diluted 10-fold (0.03 mg/ml) in 5% dextrose (Dex, pH 7.2) for SC injection (0.3 mg/kg, less than 0.3 ml/mouse). All analgesic and vehicle solutions were filter-sterilized (0.22 µm, Millipore, Bedford, MA) prior to dosing. The doses for injection and oral dosing are provided in Table 1.

Body weight and food and water intake. BW, FI, and WI were measured daily from the animal's day of arrival through approximately 2 wk of postsurgical recovery. This monitoring was performed between 0900 and 1000 each day on a top-loading balance accurate to within 0.1 g. Reported values were calculated by subtracting each day's value from the value measured the previous day. BW was corrected for transmitter weights (described later). Care was taken to correct for food spillage into the bottom of the cage, although FI may represent a slight overestimation because fine food crumbs could not be weighed. WI was determined by daily weighing of water bottles. Inadvertent water spillage during the weighing procedure was determined by simulating the weighing procedure and calculating the mean water loss in bottles that had never been placed into a cage with an animal. Based on this control measurement, inadvertent water loss from the weighing procedure was determined to be less than 0.1 ml/bottle.

T_c and activity. T_c (within 0.1 °C) and activity (counts) were collected every 5 min (10-s averages) from the transmitter device (Dataquest ART system, Data Sciences International, St Paul, MN). Each transmitter emits a unique frequency that is received by an antenna under each animal's cage and transferred to a peripheral processor connected to a personal computer. The emitted frequency is converted to T_c values by using predetermined calibration values. Activity is determined by changes in signal strength as the animal moves on the receiver board, representing a general measure that does not distinguish between locomotor movements and postural changes.

Table 1. Group characteristics for vehicle and analgesia treatments

	N	Day 0		Day 1	
		Treatment	Dose	Treatment	Dose
Surgical groups					
Bup–Indo	12	Buprenorphine	0.3 mg/kg	Indomethacin	1.0 mg/kg
Indo–Indo	11	Indomethacin	1.0 mg/kg	Indomethacin	1.0 mg/kg
Dex–SCarb	12	5% Dextrose	10.0 ml/kg	0.01 M Na_2CO_3	11.2 ml/kg
SCarb–SCarb	12	0.01 M Na_2CO_3	11.2 ml/kg	0.01 M Na_2CO_3	11.2 ml/kg
Nonsurgical groups					
Bup–Indo	6	Buprenorphine	0.3 mg/kg	Indomethacin	1.0 mg/kg
Indo–Indo	6	Indomethacin	1.0 mg/kg	Indomethacin	1.0 mg/kg
Dex–SCarb	6	5% Dextrose	10.0 ml/kg	0.01 M Na_2CO_3	11.2 ml/kg
SCarb–SCarb	6	0.01 M Na_2CO_3	11.2 ml/kg	0.01 M Na_2CO_3	11.2 ml/kg

The vehicle solutions (SCarb and Dex) were administered as volume equivalents to the active drugs. All solutions were at pH 7.2. Subcutaneous injection was performed in isoflurane-anesthetized mice immediately after (day 0) transmitter implantation (or after 15 min anesthesia exposure in animals without surgery). Oral administration was performed at 0900 the following day. The details of the injection and oral dosing procedures are provided in Materials and Methods.

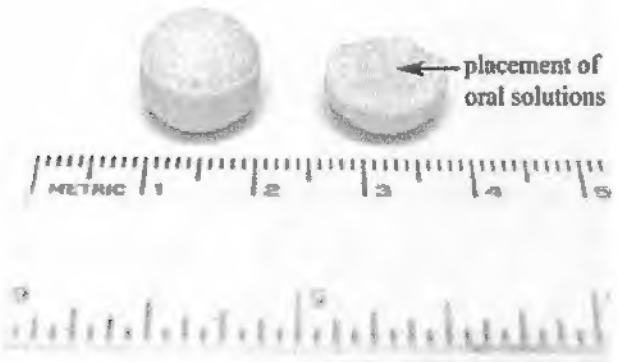

Figure 1. Photograph depicting the mini-treat used for oral administration of the vehicle and analgesic solutions. Each full treat (left side of figure) weighs 1 g with a caloric value of 3.29 kcal. Each treat was split, and 1/2 of the treat (approximately 0.5 g, right side of figure) was prepared with 70 µl of the vehicle or analgesic solution and placed on the cage floor for voluntary consumption.

All transmitters were calibrated prior to and at the completion of experimentation and were activated more than 24 h prior to implantation (as recommended by the manufacturer), to ensure measurement validity. T_c and activity values were collected in freely moving, conscious animals beginning at 1900 (start of lights-off period) on the day of surgery (day 0). Radiotelemetry data are graphed as 1-h averages, for ease of presentation. Due to a limited number of receiver boards, radiotelemetry data were collected for a subset of animals only (n = 7 to 9 per group).

Data analysis. Data are given as mean ± SEM. Group differences were analyzed using repeated-measures 2-way ANOVA and starting values of BW, FI, WI by 1-way ANOVA; both statistical analyses were followed by the Holm–Sidak test for multiple comparisons (Sigma Stat for Windows Version 3.5, Systat Software, Chicago, IL). Differences were considered to be significant when P was less than 0.05.

Results

BW, FI, and WI in surgical treatment groups. Transmitter implantation had a profound effect on BW (Figure 2 A), FI (Figure 2 B), and WI (Figure 2 C) in the 4 treatment groups. Mice weighed 21.3 ± 0.4 g (mean ± SEM) on arrival (day –24, data not shown) and gained 3.3 ± 0.1 g during the approximately 3-wk period prior to transmitter implantation. BW gain did not differ among treatment groups at any time point prior to surgery, and baseline age, BW, FI, and WI were virtually identical between treatment groups on the day of surgery (day 0, Table 2 and Figure 2). Transmitter implantation induced a 3.2 ± 0.1 g decrease in BW on day 1 that did not differ among groups. The SCarb–SCarb and Dex–SCarb vehicle control groups showed a virtually identical BW decrease of 4.0 ± 0.2 g by day 2 (open and closed circles, respectively, Figure 2 A). BW loss of the Indo–Indo (2.8 ± 0.1 g) and Bup–Indo (3.4 ± 0.1 g) treatment groups (open and closed triangles, Figure 2 A) was significantly (ANOVA, $P < 0.001$ for both groups) less than that of their vehicle controls on days 2 and 3. The BW decrease observed in the Indo–Indo group (closed triangles, Figure 2 A) was significantly (ANOVA, $P < 0.001$) less than that of all other groups on days 2 and 3. By day 4, BW gain of the SCarb–SCarb, Dex–SCarb, and Bup–Indo groups had reached that of the Indo–Indo group and remained virtually identical among all groups through day 13. Despite a steady increase from days 4 to 13, the mean BW of all treatment groups still remained below presurgical levels during the 13-d observation period (Figure 2 A; ANOVA, $P < 0.001$ for all groups).

Figure 2 B shows the effect of vehicle or analgesic treatments on changes in FI. Baseline food consumption was 3.4 ± 0.1 g in all groups during the 24 h period prior to surgery (Table 2). Transmitter implantation induced a similar reduction in food consumption during the 24-h period after surgery in all groups (day 0, 3.1 ± 0.2 g; Figure 2 B). On day 1, FI of all treatment groups showed a trend toward recovery to presurgical levels, an effect that was more pronounced in the Bup–Indo and Indo–Indo groups compared with their vehicle controls (Figure 2 B; ANOVA, $P < 0.001$). Changes in FI of the Bup–Indo and Indo–Indo groups did not differ from one another on any day of recovery. However, the effect of Bup–Indo treatment on FI recovery remained significantly ($P < 0.05$) greater than its vehicle control on day 2. FI of all treatment groups recovered to presurgical levels by day 3.

Baseline WI during the 24-h period prior to surgery (day –1) was virtually identical in all groups, at 4.6 ± 0.4 ml (Table 2). Transmitter implantation induced a 4.0 ± 0.3-ml reduction in WI during the 24-h period after surgery (day 0) that was similar in all treatment groups (Figure 2 C). On day 1, the Indo–Indo and Bup–Indo treatment groups showed greater recovery of WI than did their vehicle controls, an effect that was significantly

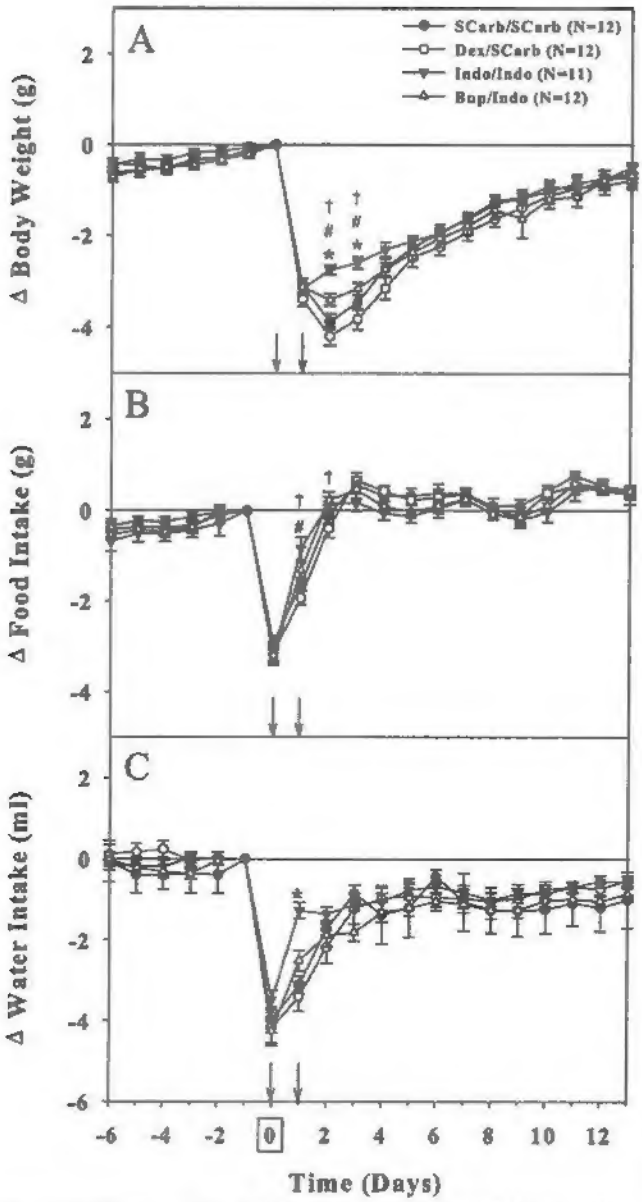

Figure 2. Changes in (A) BW, (B) FI, and (C) WI after IP implantation of a radiotelemetry device in male C57BL/6J mice receiving vehicle or analgesic treatments. The transmitter represented approximately 14% of mouse BW. Day 0 represents the day of surgery. Changes are relative to the values obtained immediately (BW) or during the 24 h (FI and WI) prior to surgery. Sample sizes are indicated in parentheses. Mice received SC injection of vehicle or analgesic on the day of surgery followed by oral dosing with vehicle or analgesic at 0900 the next day (arrows in figure). Details of the treatment groups are provided in Table 1. *, Significant ($P < 0.05$) difference between Indo–Indo and Bup–Indo groups; #, significant ($P < 0.05$) difference between SCarb–SCarb and Indo–Indo groups; †, significant ($P < 0.05$) difference between Dex–SCarb and Bup–Indo group.

(ANOVA, $P < 0.001$) more pronounced in the Indo–Indo group (Figure 2 C). Although WI was similar among groups from day 3 to 13, WI of the SCarb–SCarb and Bup–Indo groups did not return to presurgical levels within 13 d of surgery (Figure 2 C).

BW, FI, and WI in nonsurgical treatment groups. To our best knowledge, the effect of the doses of Bup and Indo used in this study have not previously been tested with respect to their effects on BW, FI, and WI in nonsurgically treated mice. Therefore, vehicle and analgesic effects on changes in BW, FI, and WI were determined in male C57BL/6J mice that were not implanted with a radiotelemetry device. Mice were weight-matched on arrival and each assigned to 1 of the 4 dosing regimens (Table 1). BW gain did not differ among groups at any time point prior to treatment, and baseline age, BW, FI and WI were virtually identical among the nonsurgical groups prior to dosing (Table 2). The Bup–Indo group showed a decrease in BW (–0.9 ± 0.2 g) on day 1 after injection that was significantly ($P < 0.001$) greater than that observed in the other treatment groups (approximately –0.1 g; Figure 3 A). BW of the Bup group remained decreased below presurgical levels through day 2 and returned to baseline on day 3. The other groups did not show a decrease of BW below presurgical levels on any day after injection.

FI did not differ between groups prior to vehicle or analgesic treatment; however, the provision of fresh food and a new cage 2 d prior to injection induced an increase in FI over baseline levels in all groups (Figure 3 B, day -2). We typically observe this response in mice after placement of fresh food into the cage.[18] On the day of injection (day 0), mice in all treatment groups significantly decreased FI below presurgical levels, although the response was significantly ($P < 0.001$) greater in the Bup–Indo compared with all other groups (Bup–Indo, –1.7 ± 0.2 versus other groups, –0.9 ± 0.1 g; Figure 2 B). By day 2, the Indo–Indo, Dex–SCarb, and SCarb–SCarb groups showed additional decreases in FI that matched the level achieved by the Bup–Indo group on day 0, such that differences between groups were no longer evident. FI in all groups remained depressed below presurgical levels from day 1 through day 4 but represented quantities normally eaten by these mice for this time period after a cage change with fresh food.

WI was virtually identical in all treatment groups prior to vehicle or analgesic injection. The injection of Bup on day 0 induced a significant ($P < 0.001$) decrease (–0.5 ± 0.1 g) in WI below presurgical levels (Figure 3 C). Despite this decrease in WI, the Bup–Indo group did not differ significantly from the other treatment groups on any day (Figure 3 C).

Circadian T_c and activity rhythms in surgical groups. A subset (n = 7 to 9) of mice from each of the surgical treatment groups was placed on receiver boards at 1900, after transmitter implantation (represented as night 0 in Figure 4), and was monitored continuously with minimal human disturbance to avoid disruptions of circadian T_c and activity rhythmicity. The mice showed the typical circadian T_c and activity pattern throughout the 13-d observation period, with low daytime values (12 h means: T_c, 36.4 ± 0.1 °C; activity, 2.9 ± 0.6 counts) and high nighttime values (12 h means: T_c, 37.0 ± 0.1 °C; activity, 5.0 ± 0.7 counts) that were similar among groups. In other words the circadian patterns of T_c and activity remained intact throughout the post-surgical period and were not impacted by the treatments. However, an analysis of 1-h values of T_c and activity revealed significant effects of analgesic treatment during the lights-on–to–lights-off transition periods. The Indo–Indo group displayed significantly (ANOVA, $P < 0.001$) greater activity than did its vehicle control group during the transition from 1900 to 2100 and 0500 to 0600 during night 1 (Figure 4 B). A similar effect was observed from 2100 to 2200 during night 2 (Figure 4 B; ANOVA, $P < 0.001$). This difference in activity levels did not correlate with circadian T_c changes, except during a 1-h period of night 1 (1900), when T_c of the Indo–Indo group was significantly (ANOVA, $P < 0.001$) elevated above control values (Figure 4 A). Circadian T_c and activity levels of these 2 groups did not differ at any other time during the 13-d observation period.

Table 2. Baseline characteristics of male C57BL/6J mice

	SCarb–SCarb	Dex–SCarb	Indo–Indo	Bup–Indo
Surgical groups				
N	12	12	11	12
Age, d	78	78	77	77
Body weight, g	25.3 ± 0.5	25.2 ± 0.5	26.0 ± 0.5	25.6 ± 0.5
Food intake, g	3.4 ± 0.2	3.5 ± 0.1	3.2 ± 0.1	3.5 ± 0.1
Water intake, ml	4.8 ± 0.5	4.6 ± 0.4	4.2 ± 0.2	4.8 ± 0.4
Nonsurgical groups				
N	6	6	6	6
Age, d	66	66	66	66
Body weight, g	22.5 ± 0.6	23.3 ± 0.5	22.9 ± 0.6	23.8 ± 0.5
Food intake, g	4.2 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	4.2 ± 0.1
Water intake, ml	3.1 ± 0.2	3.7 ± 0.3	3.0 ± 0.3	3.5 ± 0.2

Values are given as mean ± SE. Body weight represents the value measured immediately prior to transmitter implantation (surgical groups) or anesthesia exposure (nonsurgical groups) on day 0. Food and water intake values represent the amount consumed during the 24-h period prior to transmitter implantation (surgical groups) or anesthesia exposure (nonsurgical groups) on day –1. Treatment group details are described in Table 1.

Bup–Indo treatment appeared to have a greater effect on circadian T_c than on activity, compared with effects in the vehicle control (Figure 5). The Bup–Indo group showed significantly (ANOVA, $P < 0.001$) greater T_c peaks than did the Dex–SCarb vehicle controls at 2000 of night 1, 0700 of day 2, and 1900 of night 2 (Figure 5 A). Activity remained unaffected by Bup–Indo treatment (Figure 5 B), indicating dissociation between the effect of this treatment regimen on these 2 variables.

Figure 6 provides a direct comparison of Indo–Indo and Bup–Indo treatments on circadian T_c and activity rhythms. Two leading differences were noted between groups. First, the Indo–Indo group showed an earlier T_c rise in anticipation of the lights-on period from 0500 to 0600 during night 0 than did the Bup–Indo group; this effect was no longer apparent by day 2 (Figure 6 A; ANOVA, $P < 0.001$). Second, compared with the Bup–Indo group, the Indo–Indo group showed significantly (ANOVA, $P < 0.001$) greater activity from 1900 to 2000 during night 1 and 2100 to 2200 during night 2 (Figure 6 B).

Discussion

This study compared the effects of 2 analgesic regimens (Bup–Indo versus Indo–Indo) on the postsurgical recovery of mice that were surgically implanted with a radiotelemetry device. A 3.5-g transmitter was implanted into approximately 26-g mice, and the rates of recovery of BW, FI, and WI and time required for establishment of robust circadian T_c and motor activity rhythms were examined. Surgical recovery was defined as a return of BW, FI, and WI to presurgical levels and the establishment of a robust circadian profile of T_c and motor activity rhythms that did not change across consecutive days. These criteria are based on the assumption that any deviation of these variables from baseline is representative of a pathophysiologic state that could compromise animal health and well-being as well as experimental data quality and interpretation. Mice received a single dose of analgesic on 2 consecutive days, which consisted of SC injection of vehicle, buprenorphine,or indomethacin on the day of surgery followed by oral administration of vehicle or indomethacin the next day. Nonsurgical control mice, which did not receive a radiotelemetry device, received analogous drug treatments (Table 1). Transmitter implantation induced reductions in BW, FI, and WI in vehicle-treated (Dex–SCarb and SCarb–SCarb) mice; these decreases were attenuated in animals receiving Bup–Indo or Indo–Indo treatment. A direct comparison between the Bup–Indo and Indo–Indo groups indicated that Indo–Indo treatments were more effective for inhibition of these responses. In nonsurgical mice, the Bup–Indo treatment induced reductions in BW and FI, indicating adverse effects of this drug in the absence of an incisional wound or pain. These findings indicate the importance of assessing analgesic effects on these variables in nonsurgically manipulated animals to understand the nonspecific effects of these drugs under varying regimens (that is, route and dose of administration). Although we used a dose of buprenorphine (0.3 mg/kg SC) that was higher than that reported in other studies (0.05 to 0.1 mg/kg), we did not anticipate a nonspecific effect of the dose we selected, given that some studies used doses as high as 2.0 mg/kg SC without effect on the variables we measured in the present study.[7] Our unexpected findings of a nonspecific effect of buprenorphine speak to the importance of assessing a drug's effect in the absence of a surgical procedure.

The anorexic effects of the vehicle and analgesic treatments in the nonsurgical mice (Figure 3 B) appear to be confounders in our study, because we did not expect any of our treatment regimens to cause a decrease in food consumption in this group. Provision of fresh food into new cages 2 d prior to injection and its stimulation of increased FI (day –2, Figure 3 B) probably compromised this aspect of our study. Mice typically show profound increases (approximately 2-fold) in FI in response to fresh food, which requires approximately 3 d to return to baseline levels.[18] The progressive decrease in FI in the Indo–Indo, Dex–SCarb, and SCarb–SCarb groups from day 0 to day 1 is likely a representation of this pattern of FI recovery after a cage change, rather than being a direct effect of the drug treatment. That is, all mice showed a progressive decrease in FI during the days after treatment, which subsided by day 1 and although they remained at this newly depressed level throughout observation (Figure 2 B), they reflected normal baseline intakes. If the anorexic effects in the other groups were similarly due to drug treatment, we would have expected a return of FI to baseline levels during the subsequent recovery days, which did not occur. However, Bup injection induced a larger decrease in FI than did the treatments, indicating a direct negative effect of this drug on FI that exceeded the decrease normally seen in all groups in the days after fresh food provision.

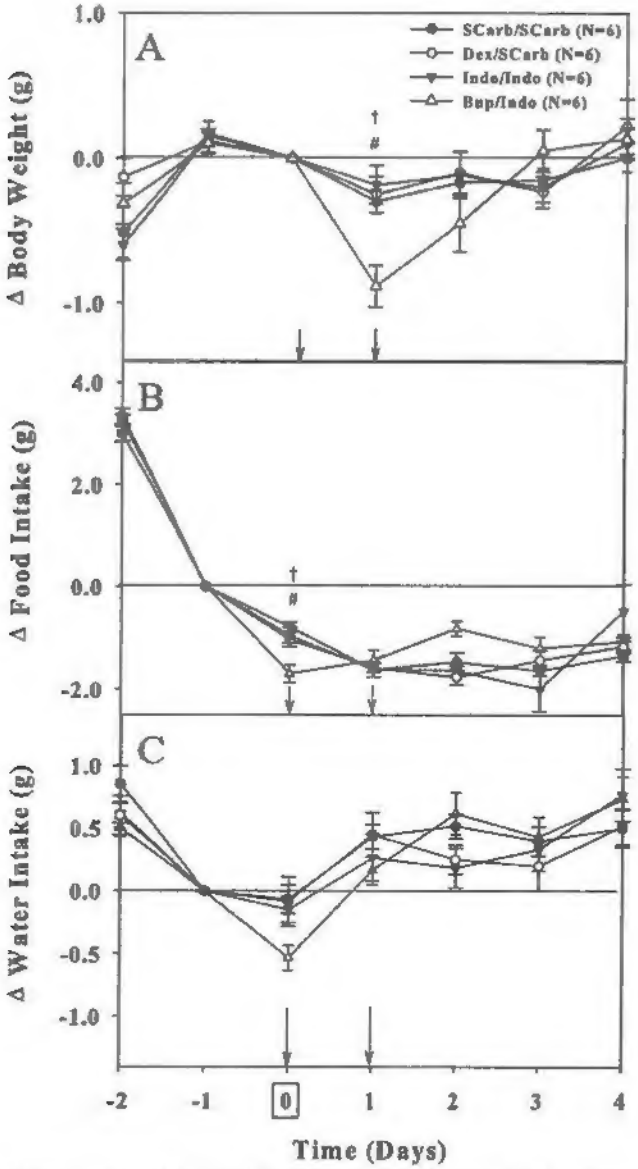

Figure 3. Changes in (A) BW, (B) FI, and (C) WI in male C57BL/6J mice receiving vehicle or analgesic treatments. Day 0 represents the day drug treatment started. Changes are relative to the values obtained immediately (BW) or during the 24 h (FI and WI) prior to surgery. Sample sizes are indicated in parentheses. Mice received SC injection of vehicle or analgesic on the day of surgery followed by oral dosing with vehicle or analgesic at 0900 the next day (arrows in figure). Details of the treatment groups are provided in Table 1. *, Significant ($P < 0.05$) difference between Indo–Indo and Bup–Indo groups; #, significant ($P < 0.05$) difference between SCarb–SCarb and Indo–Indo groups; †, significant ($P < 0.05$) difference between Dex–SCarb and Bup–Indo group.

Typically, FI and WI are correlated in rodents. In our data, the large increase in FI on day –2 was paired with an increase in WI (compare day –2 data in Figures 3 B and 3 C). Bup induced a decrease in WI below baseline levels, which was not observed in the other treatment groups. The resumption of FI to the new 'below-baseline' levels on days 1 to 4 was matched with a return of WI to baseline on these days. Therefore Bup appears to have nonspecific effects on FI and WI that are not related to pain and that were not similarly associated with the other treatment regimens. A potential complicating factor with respect to nonspecific effects of opioids is that the effects have been reported to differ depending on the presence (or absence) of pain, which we did not assess in this study. In a previous study,[22] 2 doses (0.05 mg/kg SC) of buprenorphine given 9 h apart induced a greater decrease in food consumption among rats that were not surgically manipulated than in animals that received buprenorphine after surgery. These data indicate that the effects of buprenorphine on food intake may be attenuated in animals presumed to be experiencing postsurgical pain. Further complicating issues include the route of administration, dose, and dosing frequency as well as the species, size, and age of the animal, all of which can influence nonspecific opioid effects related to feeding and drinking.[7,13,22,23,28,35] Of particular importance regarding the use of buprenorphine is the frequency of dosing. As previously shown, multiple doses often induce anorexic effects whereas a single dose may avoid these effects.[7,28]

Sedation is a term used to describe the diminishment of behaviors such as feeding, drinking, and activity, and has often been associated with opioids. Buprenorphine may, as mentioned earlier, reduce feeding and drinking behaviors, but when it comes to locomotor activity, most studies have found the opposite.[7,12,27,28] General levels of activity, even in pain-free animals, often are increased in buprenorphine-treated rodents.[12,26,27] In the current study, we observed increased activity levels in the Indo–Indo group during the lights-on–to–lights-off transition periods that were not present in the Bup–Indo group. Given that indomethacin has not been reported to induce hyperactivity, we conclude that the increased activity level of the Indo–Indo group relative to that of the Bup–Indo group is an indication of reduced postsurgical pain and not the result of buprenorphine-associated sedation effects, suggesting enhanced analgesic efficacy by indomethacin.

With respect to circadian T_c and motor activity profiles, only minor changes occurred with analgesia treatment and primarily were reflected as higher levels of motor activity in the Indo–Indo versus Bup–Indo group during the transition to the lights-off or active night period. Taken together, our data suggest that a potent NSAID, such as indomethacin, may be more efficacious for postsurgical recovery in radiotelemetry-equipped mice than is an opioid analgesic such as buprenorphine.

The relationship between pain and surgical recovery, although not quantifiable, is logically an inverse one, because objective measures of recovery are anticipated to improve with decreasing pain. Therefore reducing postoperative pain becomes important, because doing so likely will accelerate recovery, improve the quality of subsequently collected experimental data, and provide comfort to animals.

In addition to the type of analgesic, the extent and length of pain relief for a given species or surgical procedure are well known to be dependent on the dose, frequency, and route of administration. Our choices of analgesics, dosing regimen, and route of administration reflected several factors. We chose to compare responses between buprenorphine and indomethacin because of their differing mechanisms of pain relief. Buprenorphine is an opioid analgesic that blocks pain signal transmission (μ-receptor agonist function) and pain sensation (κ-receptor antagonist function) by the brain.[17] The analgesic efficacy of buprenorphine has been demonstrated in rats and mice across a broad range of analgesia challenges in models of acute and chronic pain and currently represents the most widely used analgesic in rodent studies.[3] Unfortunately, the wide use of buprenorphine in mouse models of surgery persist, despite few objective assessments that confirm its superiority for postsurgical recovery in this species. Indomethacin is a cyclooxygenase

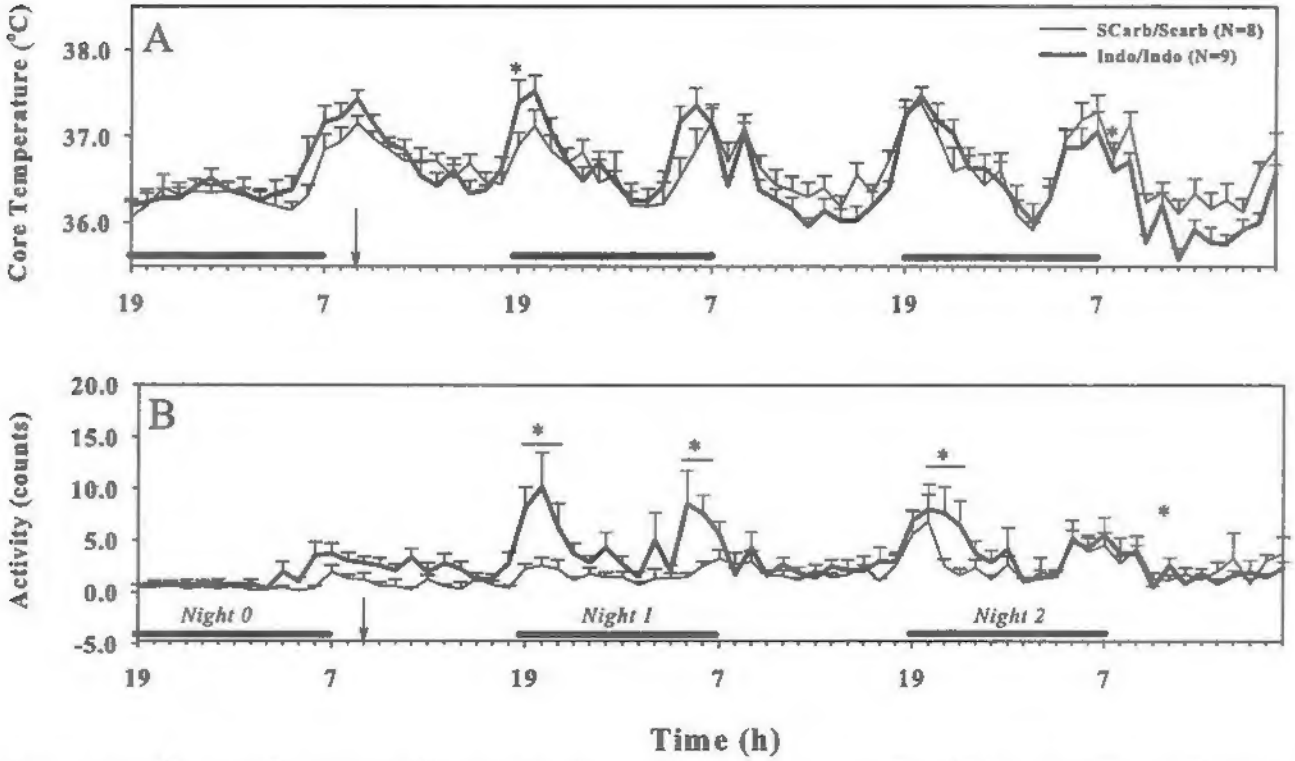

Figure 4. Comparison of vehicle and analgesic (Indo–Indo) effects on (A) core temperature and (B) activity of male C57BL/6J mice after IP implantation of a radiotelemetry device. The transmitter represented approximately 14% of mouse BW. Mice received SC injection of vehicle or analgesic on the day of surgery (not shown) followed by oral dosing with vehicle or analgesic at 0900 the next day (arrows in figure). Details of the treatment groups are provided in Table 1. Sample sizes are shown in parentheses. Data represent 1-h averages and are only presented through day 3 because circadian profiles did not change on subsequent days. Black horizontal bars represent the lights-off (active; 1900 to 0700) period during a 12:12-h photoperiod. *, Significant ($P < 0.05$) difference between groups.

inhibitor that possesses analgesic and antiinflammatory properties, which are due to its inhibitory effects on prostaglandin production. Prostaglandin production is correlated directly to pain perception because prostaglandins lower the threshold for the activation of nociceptors; therefore, indomethacin functions by blocking nociceptor sensitization under conditions of injury and inflammation.[32]

To compare the efficacies of Bup–Indo versus Indo–Indo treatment regimens directly, mice received SC injection on the day of surgery with a novel oral method of indomethacin delivery on a flavored treat that permitted voluntary consumption on the following day. Although one of the objectives of this study was to develop a noninvasive method of analgesic administration (for example, voluntary consumption) to reduce animal stress, we were unable to provide mice oral buprenorphine on either day of treatment because of the high first-pass metabolism of this drug by the liver (approximately 95%), which markedly limits the drug's systemic bioavailability.[2,23] Furthermore, the doses of oral buprenorphine (5 to 10 mg/kg) required to achieve systemic concentrations that provide analgesic efficacy similar in magnitude to that achieved after SC injection are associated with several adverse side effects, including lack of palatability, pica (a perverted appetite for nonfood items, such as bedding), and unacceptable behavioral and gastrointestinal effects.[24,35] The complications associated with high doses and first-pass metabolism of buprenorphine resulted in the use of oral indomethacin treatment for both analgesia groups on the day after surgery. Therefore, we did not determine the potential efficacy of multiple buprenorphine treatments on postsurgical recovery, although other studies have examined this issue.[13,34]

We also included nonsurgical control groups that received the same drug treatments (and anesthesia) to determine whether the treatments alone caused anorexia. Although a previous study[7] showed no effect on anorexia of mice of a single injection of buprenorphine at a dose approximately 7-fold higher than that we used, we saw significant decreases in BW (day 1) and FI (day 0) in mice that received Bup–Indo compared with those that received Indo–Indo or vehicle. These surprising results suggest that even a single therapeutic dose (0.3 mg/kg SC) of Bup can have negative consequences that likely would delay surgical recovery in the short-term. A lower therapeutic dose (0.05 or 0.1 mg/kg) might blunt these effects.

The effectiveness of oral consumption of NSAIDs has been questioned, because drinking behavior is often negatively affected by the adverse taste or smell of the solution.[33] However, we developed a novel method for oral consumption of indomethacin for the second day of treatment by pipetting the solution onto a pungently flavored treat that masked any odor that may have been associated with the drug; this method proved to be very effective, as mice rapidly consumed the treat on its placement on the cage floor. Although NSAID treatment was provided during postsurgical recovery in the current study, a similar method could be used for presurgical NSAID treatment to avoid the problems associated with provision of these solutions in the drinking water. Although we did not note an adverse effect of postsurgical NSAID treatment on wound healing in our model, presurgical or prolonged postsurgical treatment with these drugs might impair the clotting cascade, potentially impeding postsurgical wound healing.

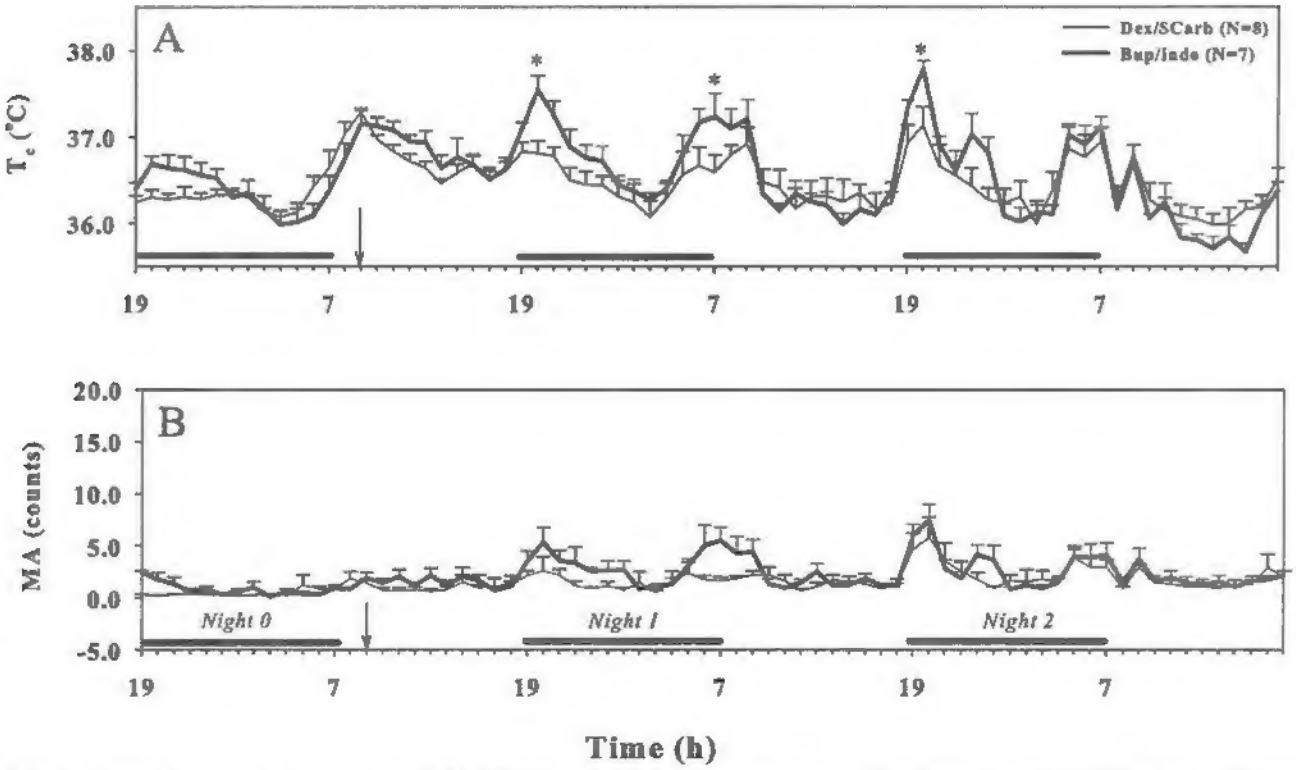

Figure 5. Comparison of vehicle and analgesic (Bup–Indo) effects on (A) core temperature and (B) activity of male C57BL/6J mice after IP implantation of a radiotelemetry device. The transmitter represented approximately 14% of mouse BW. Mice received SC injection of vehicle or analgesic on the day of surgery (not shown) followed by oral dosing with vehicle or analgesic at 0900 the next day (arrows in figure). Details of the treatment groups and dosing regimen are provided in Table 1. Sample sizes are shown in parentheses. Data represent 1-h averages and are only presented through day 3 because circadian profiles did not change on subsequent days. Black horizontal bars represent the lights-off (active; 1900 to 0700) period during a 12:12-h photoperiod. *, Significant ($P < 0.05$) difference between groups.

The decision to provide 2 d of analgesia treatment was based on the duration of pain relief we thought would be needed after the major surgical procedure required for transmitter implantation. Although we did not directly measure pain in this study, previous studies in rats have done so. One study evaluating the duration of clinical effects and hyperalgesia in rats that experienced abdominal surgery concluded that postoperative pain was present for the first 24 h, the 'acute postoperative phase'.[4] Due to prolonged reductions in BW (approximately 14 d), FI (2 to 3 d), and WI (more than 14 d) that we typically observe in radiotelemetry-equipped mice,[19] we chose an analgesic treatment strategy that was directed toward the first 48 h of recovery, with the doses of buprenorphine (0.3 mg/kg) and indomethacin (1 mg/kg) representative of those within the demonstrated therapeutic window for pain relief by these classes of drugs.[20,34]

One area of potential weakness within our analgesia regimen design was the frequency of treatment. Given an estimated length of pain relief of between 6 and 12 h for both buprenorphine and indomethacin, blood levels of the analgesics may have fallen below those that would provide sufficient analgesia until the follow-up dose the next morning. We since have modified the indomethacin formula by dissolving it in 2-hydroxypropyl β cyclodextrin (pH 7.2). This solvent provides several advantages over that used previously, 0.01 M sodium carbonate (pH 7.2). Hydrophilic cyclodextrins greatly increase the solubility of drugs (that is, indomethacin) in aqueous solutions, often provide protection from premature drug degradation, and can extend therapeutic blood levels of many drugs.[36] Although these benefits might suggest that a longer drug-dosing interval would be possible, in the interest of ensuring effective analgesia relief, it would be best not to make this assumption. Furthermore, because in this study we did not use any pharmaceutical enhancers, the interval of postsurgical analgesic administration we used (18 to 24 h) likely could have been improved by shortening it to no more than 12 h. Despite this potential shortcoming, the results of this study provide relevant information with regard to a potential alternative to buprenorphine, because all groups received treatment at the same interval, thereby allowing for direct comparisons of the measured indices of surgical recovery. Our data suggest that indomethacin may be more efficacious than buprenorphine relative to surgical recovery in mice and can be delivered in a humane way (voluntary consumption of an oral treat) that minimizes stress. An alternative strategy would be to use a different, more commonly used NSAID, such as carprofen (for example, Rimadyl), for pain relief in postsurgical mice. Carprofen has only a modest blood-thinning effect, can be administered SC or PO, and has been shown to be an effective postsurgical analgesic in rats.[22] Carprofen may very well prove to be a good alternative to buprenorphine for postsurgical analgesia after radiotelemetry implant procedures with mice, although this analgesic has not been tested in our laboratory.

In previous studies, we showed that C57BL/6J male mice require approximately 14 d to recover presurgical levels of BW after IP implantation of a radiotelemetry device (approximately 15% body mass) when analgesia is not provided.[19] This time course of recovery is virtually identical to our current study, in which animals were treated with Bup–Indo and Indo–Indo. Therefore, although analgesic treatments in this study were efficacious during the early stages of recovery (through day 3),

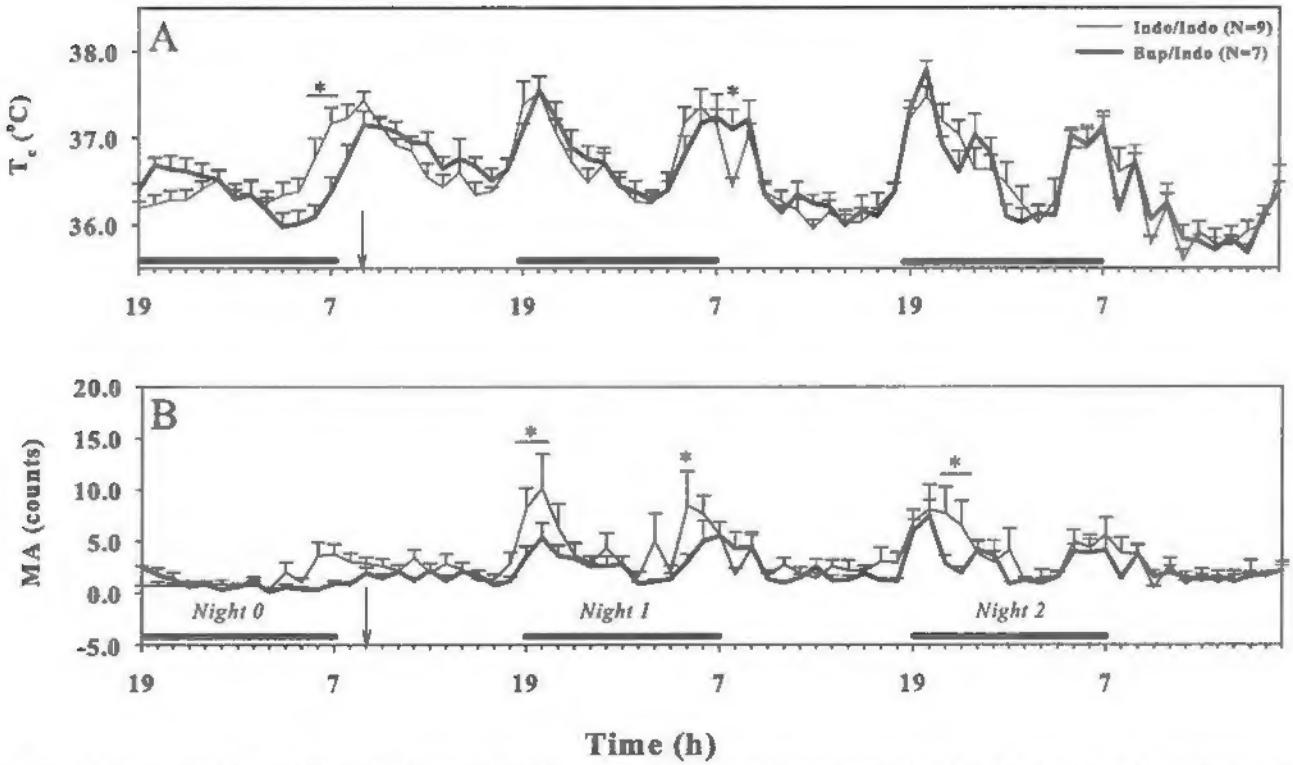

Figure 6. Comparison of analgesic (Bup–Indo versus Indo–Indo) effects on (A) core temperature and (B) activity of male C57BL/6J mice after IP implantation of a radiotelemetry device. The transmitter represented approximately 14% of mouse BW. Data represent the treatment groups shown in Figures 4 and 5 for direct comparison. Data are only presented through day 3 because circadian profiles did not change on subsequent days. Black horizontal bars represent the lights-off (active; 1900 to 0700) period during a 12:12-h photoperiod. *, Significant ($P < 0.05$) difference between groups. Details of the dosing regimen are provided in Table 1.

mice still required approximately 14 d to return to presurgical BW (Figure 2 A). Note that BW remained depressed on days 4 through 13, despite the resumption of normal food and water consumption patterns. This effect may have been related to the relatively large size of the radiotelemetry implant and may be improved with the use of a smaller device for remote T_c sensing in this species. We recently tested this hypothesis by comparing postsurgical recovery rates of Indo-treated C57BL/6J male mice implanted with large (approximately 3.5 g) versus small (approximately 1.1 g) transmitters; the implantation of a smaller device (approximately 4% body mass) was associated with an accelerated rate of recovery (approximately 3 d) for BW and normal FI levels (WI was still significantly attenuated).[16] These data reinforce our previous recommendation of using 1 to 2 d of analgesia treatment in conjunction with the smallest instrumentation possible as the optimal approach for improvements in health and physiologic functioning in surgically manipulated mice.[19]

One of the goals of the present study was to develop techniques for assessment of postsurgical pain, recovery, and analgesia treatment that are easily implemented into research protocols while introducing little or no handling stress to the animal. Therefore, we used daily changes in BW, FI, and WI as objective yet indirect measures of postsurgical pain and distress rather than measuring pain directly with traditional hyperalgesia technologies (for example, tail-flick test). The main assumption of our approach was that postsurgical pain and distress would contribute to decreased food and water consumption (with consequent effects on BW). Laparotomy introduces a large peritoneal wound that could be irritated by caging systems that require the mouse to reach up to the cage lid to obtain food and water. The fact that on the day after surgery all mice voluntarily consumed the Supreme Mini-Treat on its placement on the cage bottom indicates the applicability of this method for analgesic administration with minimization or elimination of animal handling. Our choice of a pungently flavored treat was based on our desire to mask any potential aversive smell of indomethacin; although we were not able to confirm the effectiveness of this strategy for odor masking, the provision of a pungent food source or perhaps mashed food on the cage bottom may be an effective technique to improve postsurgical food consumption in this species. Regardless of the mechanism, this novel method of treatment was effective for drug delivery and greatly improved our experimental design by eliminating the need for an injection procedure in mice with an extensive incisional wound.

Because this study used a radiotelemetry device to assess postsurgical recovery rates in mice, we monitored circadian T_c and motor activity profiles as additional objective measures of analgesic efficacy. We observed short-duration (1 or 2 h) elevations in T_c (Bup–Indo) or activity (Indo–Indo) in the analgesic-treated animals compared with their vehicle controls during the transitions between the lights-on (inactive) and lights-off (active) periods. The presence of higher motor activity levels in the Indo–Indo groups at these time points is suggestive of a faster rate of recovery in these mice, as postsurgical pain and distress is expected to suppress motor activity levels. The mice treated with Bup–Indo showed virtually identical levels of motor activity as those of their vehicle controls, which were significantly depressed during the first 3 nights of recovery (Figure 5). This decrease in activity is a typical response to transmitter implantation in this species.[19] The low activity levels

of the Bup–Indo groups correlated with significantly elevated T_c values, which may be indicative of a febrile response to inflammation. However, several observations do not support a fever hypothesis. First, injury- and inflammatory-induced elevations of T_c typically occur during the lights-on (inactive) period.[19] Second, the T_c elevations we noted were transient in nature, being present only for 1 or 2 h; this duration is unrepresentative of fever, which typically is maintained for 4 to 8 h under the injurious conditions induced by transmitter implantation.[19] Third, prostaglandins are considered to be key mediators of fever,[30] yet indomethacin treatment on day 2 in the Bup–Indo group did not mitigate the T_c elevation (Figure 5); however we did not measure prostaglandin levels in this study.

Finally, the environmental temperature at which mice were housed may be an additional factor accounting for the long postsurgical recovery rates in the current study. Mice were housed at an ambient temperature of approximately 25 °C, which is considered a mild cold stress in this species. Due to the high ratio of surface area to body mass in mice, housing at this ambient temperature may have induced increased metabolic demands for the maintenance of T_c homeostasis. The thermoneutral zone, which corresponds to a range of ambient temperatures associated with minimal metabolic rate, ranges from 26 to 34 °C in mice,[8] suggesting that the chosen ambient temperature for this study was close or equivalent to the lower critical threshold for metabolic stimulation. Although the mice in this study may have been acclimated to 25 °C due to housing at this ambient temperature for 2 wk prior to surgery, whether housing at an ambient temperature within the thermoneutral zone would have facilitated a faster postsurgical recovery in the presence (or absence) of analgesia is unclear. Many animal facilities may not be able to achieve ambient temperature ranges in the thermoneutral zone; in such situations providing an external heating source (for example, heating pad) at least during the initial stages of postsurgical recovery to reduce the metabolic energy demands of mice presumably would be beneficial.

Although inherently complicated, the management of postsurgical pain for experimental animals is a statutory and ethical requirement as well as in the best interest of research because minimizing pain after surgery improves data quality by accelerating recovery. In this study we compared the postsurgical effects of 2 drug regimens, Indo–Indo (an analgesic with an antiinflammatory component) and Bup–Indo (an opioid analgesic and an analgesic–antiinflammatory), to determine which of these treatments is more efficacious for postsurgical recovery in mice. The data from 5 objective indices of surgical recovery suggest that, after IP implantation of a radiotelemetry device in male C57BL/6J mice, the antiinflammatory–analgesic Indo–Indo provided greater efficacy for postsurgical recovery than did a Bup–Indo combination. However, our results are specific to male C57BL/6J mice and may not be the same for other strains of mice or genetic knockouts.

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The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense.

In conducting the research described in this report, the investigators adhered to the *Guide for Care and Use of Laboratory Animals* as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

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